

E. 6 Preparing of competent cells with chaperones

For further information read the TaKaRa product manual.

No.	Plasmid	Chaperone	Protein size [kDa]	Promoter	Inducer	Res.
C1	pG-KJE7	dnaK dnaJ grpE	70 kDa 40 kDa 22 kDa	<i>araB</i>	L-Arabinose	Cm
C2	pGro7	groES groEL	10 kDa 60 kDa	<i>araB</i>	L-Arabinose	Cm
C3	pG-KJE8	dnaK dnaJ grpE	70 kDa 40 kDa 22 kDa	<i>araB</i>	L-Arabinose	Cm
		groES groEL	10 kDa 60 kDa	<i>Pzt-1</i>	Tetracycline	
C4	pTf16	tig	56 kDa	<i>araB</i>	L-Arabinose	Cm
C5	pG-Tf2	groES groEL tig	10 kDa 60 kDa 56 kDa	<i>Pzt-1</i>	Tetracycline	Cm

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Niels

Transformation of BL21 + Jm109 with Chaperone Plasmid Set (TaKaRa)

Each cell type was transformed with one of the five different plasmids

50µL cells + 1µL plasmid

Cells plated on 2xYT with Ca

Inoculated with 2xYT+Ca over night

Expression of Chaperones

Plattenlayout

Chaperone

		1	2	3	4	5	6
JM109	A	C1	C2	C3	C4	C5	(-)
	B						
BL21	C	C1	C2	C3	C4	C5	(-)

green= 2xYT+4 mg/mL Arabinose (50mL+200mg arabinose)

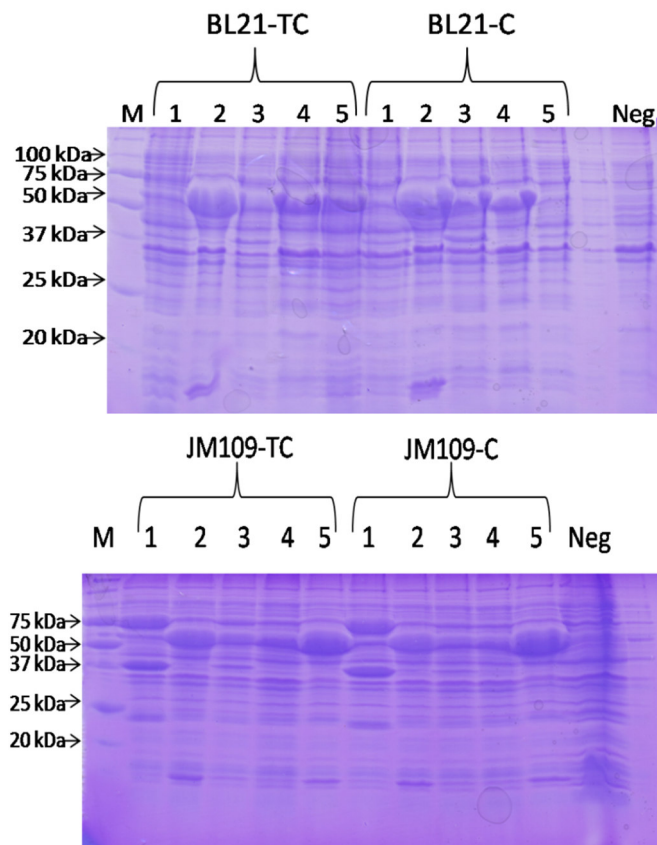
red=2xYT+4 mg/mL Arabinose+100ng Tetracyclin

Incubation: 6h ,700 rpm and 37°C

SDS-Pages of Chaperones

TC: 100 ng of Tetracycline

C: 200 ng of Tetracycline



No.	Plasmid	Chaperone	size [kDa]
1	pG-KJE7	dnaK dnaJ grpE	70 kDa 40 kDa 22 kDa
2	pGro7	groES groEL	10 kDa 60 kDa
3	pG-KJE8	dnaK dnaJ grpE groES groEL	70 kDa 40 kDa 22 kDa 10 kDa 60 kDa
4	pTf16	tig	56 kDa
5	pG-Tf2	groES groEL tig	10 kDa 60 kDa 56 kDa

→ SDS-PAGE shows that all chaperones can be expressed. The produced chemical competent cells are used for further coexpression studies with His-constructs and Lac-end.